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Sirtuins and Accelerated Aging in Scleroderma

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Abstract

Purpose of Review—Premature activation of aging-associated molecular mechanisms is emerging as an important contributor to many diseases, including scleroderma. Among central regulators of the aging process are a group of histone deacetylases called sirtuins (SIRT). Recent findings implicate these molecules as pathophysiological players in scleroderma skin and lung fibrosis. The goal of this article is to review recent studies on the involvement of SIRT in scleroderma from the perspective of aging-related molecular mechanisms.

Recent Findings—Despite a degree of controversy in this rapidly developing field, the majority of data suggest that SIRT levels are decreased in tissues from patients with scleroderma compared to healthy controls as well as in animal models of scleroderma. Molecular studies reveal several mechanisms through which declining SIRT levels contribute to fibrosis, with the most attention given to modulation of the TGF- β signaling pathway. Activation of SIRT in cell culture and in animal models elicits antifibrotic effects.

Summary—Declining SIRT levels and activity are emerging as pathophysiological contributors to scleroderma. Restoration of SIRT may be therapeutic in patients with scleroderma.

Keywords

scleroderma; systemic sclerosis; SSc; fibrosis; sirtuin; SIRT; aging; skin; lung; fibroblast

Introduction

The historically recent, global rise in life expectancy has increased the societal, economic, and human burden of aging-associated diseases and intensified research into mechanisms of aging. Aging is a complex process with distinct organismal, tissue, and cellular features, but is ultimately driven by changes at the molecular level [1, 2]. Studies in molecular

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Conflicts of Interest

The authors declare that they have no conflicts of interest with the contents of this article.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

mechanisms of aging pave the way for future strategies to improve quality of life during normal aging and treat aging-related diseases.

Molecular processes that drive normal aging may occur prematurely and contribute to diseases in a “segmental” fashion, eliciting some, but not all commonly appreciated features of aging such as wrinkled skin, grey hair, atherosclerosis, and osteoporosis [3]. Premature and segmental activation of aging may contribute, among other pathologies, to autoimmunity [4], vascular abnormalities [5, 6], and fibrosis [7–17]. It is therefore not surprising that scleroderma, or systemic sclerosis (SSc), a disease characterized by the triad of autoimmune activation, vasculopathy, and fibrosis, has been described as a disease of accelerated aging [17]. The peak incidence of SSc occurs between ages 45 and 64 [18]; greater age at disease onset is a predictor of worse survival [19]; and systemic involvement such as cardiac, pulmonary, or renal disease is more common in elderly patients [20].

Dermal and systemic, especially pulmonary, fibrosis is a particularly challenging aspect of SSc to manage and remains incompletely understood despite decades of research. Multiple molecular processes have been shown to contribute to excess extracellular matrix deposition in SSc and other fibrotic diseases, yet a cure for fibrosis remains elusive. Recent studies have shown shared molecular pathways involved in aging and the development of fibrosis in multiple organs, offering a new perspective on fibrosis and possibility for developing better therapies. Sirtuins (SIRT6), a group of histone deacetylases that require NAD for their catalytic activity [21, 22], occupy a central role among molecular mediators of aging by acting through diverse mechanisms on a broad array of molecular pathways. Recent studies have begun to explore the contribution of SIRT6 to SSc, particularly its fibrotic component. In this article, we review recent findings on aging-related mechanisms in the pathogenesis of SSc, with a focus on SIRT6 in dermal and pulmonary fibrosis.

Molecular mechanisms of aging

A comprehensive review of aging, a voluminous and highly complex area of research, is beyond the scope of this review. Here, we highlight themes from the aging literature that pertain to SIRT6, SSc, and fibrosis. Aging, the time-dependent functional decline affecting most living organisms, is a complex process resulting from the accumulation of molecular, cellular, and organ changes, leading to increased susceptibility to disease and death [1, 2, 23]. Cellular and molecular disturbances associated with aging include epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, genomic instability, and telomere attrition [1, 2]. It is important to note that many of these processes are inter-related and impact on each other, resulting in a loss of cellular homeostasis on multiple levels. Aging-associated mitochondrial damage may promote increased oxidative stress, which in turn contributes to genomic instability, loss of proteostasis, and cellular senescence.

Many of these cellular and molecular processes have emerged in studies on fibrosis in SSc and idiopathic pulmonary fibrosis (IPF), another fibrotic disorder of premature aging. Aging is associated with cumulative damage to macromolecules (DNA and protein) resulting from

a decline in DNA and protein repair mechanisms [24–26] as well as increased reactive oxygen species (ROS) driven by mitochondrial dysfunction, cellular inflammatory responses, and functional impairment of proteolytic systems [27, 28]. Oxidative stress is increased in SSc [29] and contributes to fibrosis in SSc and IPF through multiple mechanisms involving oxidative protein and DNA damage [30, 31].

Oxidative stress has been shown to contribute to cellular senescence in SSc [32]. Cellular senescence is characterized by the permanent arrest of cell proliferation, induction of the senescence-associated secretory phenotype (SASP), and selective degradation of specific proteins by the ubiquitin-proteasome pathway [33, 34]. Cellular senescence may be induced by multiple metabolic, cellular, and genotoxic stressors such as excessive ROS, DNA damage, oncogene activation, short telomeres, low NAD⁺/NADPH ratios, dysfunctional mitochondria, autophagy inhibition, and decreased ribosome biogenesis. Recent studies have linked oxidative stress and cellular senescence in SSc through the ROS-generating NADPH oxidases, in particular Nox-4, which is elevated in SSc fibroblasts [30] and contributes to fibrosis in aged mice and IPF by rendering fibroblasts senescent and resistant to apoptosis [31]. Autophagy and mitochondrial dysfunction, two other inter-related aging processes, have been implicated in SSc and other fibrotic diseases. Autophagy, a cellular proteostatic mechanism that targets damaged proteins and organelles for lysosomal degradation, and mitochondrial function both decline with aging, and impairments contribute to fibrosis in SSc and IPF as well as in animal models (see [17] for a detailed review).

Multiple molecules have been shown to regulate lifespan. NAD, the oxidized form of nicotinamide adenine dinucleotide, extends lifespan in yeast [35], restores glucose tolerance in aged mice [36], and was recently shown to increase mouse lifespan as well as muscle stem cell number, muscle regeneration, and mitochondrial function in aged mice [37]. NAD declines during aging, and its restoration mediates protein-protein interactions required for DNA repair [26]. Siglecs, sialic acid-binding immunoglobulin-like receptors expressed by immune cells, block pro-inflammatory cascades and when deficient in mice, result in increased oxidative damage, reduced lifespan, and premature aging phenotypes [27]. Mice deficient in the protein klotho show signs of premature aging [38], and serum klotho levels were recently shown to be significantly lower in patients with SSc compared to age-matched controls [39, 40].

Sirtuins are considered among the most promising targets for modulating aging-associated cellular and molecular processes and disease pathologies [41]. They have been shown to promote longevity in species across multiple taxonomic groups in response to caloric restriction or activation with molecules such as resveratrol [35, 42–47]. Multiple studies have shown declines in SIRT activity or levels with age in different organisms, tissues, and cells types [41, 48–52]. SIRT6 and SIRT7 knockout mice display progeroid phenotypes and have shorter lifespans than controls [53, 54], and SIRT3 polymorphisms have been associated with longevity in human populations [55]. Sirtuins play a protective role in multiple aging-related diseases including neurodegenerative disorders, hearing loss, cardiovascular disease, and disorders of glucose and lipid metabolism [21, 56, 57]. The seven mammalian sirtuins (SIRT1–7) share a highly conserved NAD-binding catalytic domain and are expressed in multiple organs [58], but differ widely in their biologic

functions based on different catalytic mechanisms and protein targets and localization to discrete subcellular compartments [56, 57, 59]. Although their classic function is to regulate gene transcription through deacetylation of histones, they have been shown to target protein substrates other than histones [60, 61] and have catalytically-independent effects [62].

Dietary or caloric restriction has been shown to extend lifespan in organisms such as yeast, worms, flies, and mice though inhibition of highly-conserved nutrient signaling pathways. These include the insulin-like growth factor (IGF)/insulin, mammalian target of rapamycin/ribosomal protein S6 kinase (mTOR/S6K), AMP-activated protein kinase (AMPK), RAS, and AKT/protein kinase B (AKT/PKB) signaling pathways [63]. Although the extent to which SIRT6 directly influence aging in mammals remains unclear [2, 23], SIRT6 may promote longevity in response to activation by caloric restriction by modulating these pathways and downstream cellular stress responses such as the heat shock response, endoplasmic reticulum stress, autophagy, apoptosis, ribosome biogenesis, and protein translation [23]. These effects likely result from SIRT6 interactions with multiple signaling molecules and transcription factors including Forkhead box O1 and O3 (FOXO1 and FOXO3) [52, 64–68], AMPK [64, 69], mTOR [70, 71], hypoxia inducible factors [62], and heat shock factor 1 (HSF1) [72]. Many of these very pathways and molecules such as IGF [73], AMPK [74], and mTOR [75–77] have been mechanistically implicated in the pathogenesis of SSc and other fibrotic diseases and may regulate changes in fibrosis independently or in concert with SIRT6.

In addition to regulating metabolism, SIRT6 also participate in DNA repair through multiple mechanisms including telomere maintenance [78, 79], recruitment and stabilization of DNA repairing enzymes [80], and attenuation of DNA damage in response to genotoxic stress [81]. As cellular energy sensors, SIRT6 link metabolism and DNA repair, two central inter-dependent aspects of aging [82, 83], and SIRT6 activation by NAD or caloric restriction may promote longevity in part by augmenting DNA repair mechanisms. SIRT6 may also link metabolism, DNA repair, and aging by regulating mitochondrial functions, either onsite by protecting cells from oxidative stress in mitochondria (SIRT6 3–5) [57], or remotely by regulating transcription of mitochondrial ribosomal proteins and translation factors (SIRT6) [84]. SIRT6 also regulate autophagy, another critical aging-associated process that modulates cellular stress responses [85].

In summary, SIRT6 modulate highly conserved metabolic pathways and protect cells from multiple stressors—oxidative, genotoxic, proteostatic—associated with aging. SIRT6 levels and enzymatic activation decline during normal aging, and loss of their protective effects contributes to multiple aging-associated pathologies.

Sirtuins and tissue fibrosis

Aging and fibrosis share molecular mechanisms in multiple organs, including the lung [7, 8], heart [9, 10], vascular wall [11], kidney [12], liver [13, 14], and skin [15, 16]. Accumulating literature indicates that SIRT6 regulate pathways and molecules involved not only in aging, but also in tissue and organ fibrosis (Table 1).

With occasional exceptions, the common theme of these numerous reports is the link between declining SIRT levels and development of fibrosis (mirroring the established link between SIRT decline and aging) as well as a protective, antifibrotic role of SIRT restoration (mirroring the anti-aging effect of SIRT activation or overproduction). SIRT activation has occasionally been observed to have a profibrotic outcome, which may be explained by a dose-dependent effect, with mild to moderate SIRT elevation being protective against aging and fibrosis, but exceedingly high levels being deleterious [86, 120].

Sirtuins have been shown to exert antifibrotic effects through modulation of numerous fibrosis-mediating pathways, some of which are listed in Table 2. In some cases, the observed mechanistic effects of SIRTs are so diverse, that it is difficult to narrowly classify them [69, 88, 91, 120]. Specific intracellular signaling molecules or pathways interacting functionally with or targeted directly by SIRTs include adenosine monophosphate-activated protein kinase (AMPK) – angiotensin-converting enzyme 2 (ACE2) signaling [69, 104], peroxisome proliferator-activated receptor (PPAR) γ [97], signal transducer and activator of transcription (STAT)3 [108], mammalian target of rapamycin (mTOR) [99, 102], nuclear factor erythroid 2-related factor 2 (Nrf2) [91, 117], Krüppel-like factor (KLF)15 [110], forkhead fox O1 (FOXO1) [66] and O3 (FOXO3) [67, 68], nuclear factor κ B (NF- κ B) [115], Notch1 [95], matrix metalloproteinase (MMP)-14 [92], manganese superoxide dismutase (MnSOD) [65, 87], and c-Jun [13].

We discuss in greater detail below regulation by SIRTs of some of these pathways and molecules pertaining specifically to the development of skin and lung fibrosis in SSc.

Sirtuins and scleroderma

At the time of preparation of this review, there are only a handful of reports that have directly addressed the involvement of SIRTs in SSc [99–102, 111, 112, 121]. Most have focused on SIRT1 [99–102], but literature is emerging on the role of SIRT3 [111, 112] and SIRT7 [121]. The prevailing message from these studies is that SIRTs play an antifibrotic role in SSc and engage in mutual negative feedback with the TGF- β signaling pathway, the most potent known driver of fibrosis. Some of these works have also drawn attention to the contribution of aging-related mechanisms to the development of SSc such as oxidative stress, mTOR signaling, mitochondrial damage, and cellular senescence. We discuss these studies below and include articles on SIRT regulation of skin and lung fibrosis in patients with other diseases.

Sirtuin expression in skin and lung fibrosis

The majority of studies have shown decreased SIRT expression in tissues and fibroblasts from patients with SSc compared to controls and in experimental fibrosis in mice [99–101, 111, 112, 121]. Decreases in SIRT1 were observed in fibrotic skin and dermal fibroblasts from patients with SSc [100, 101] and in peripheral blood mononuclear cells of SSc patients with pulmonary fibrosis compared to patients without fibrosis [99]. Echoing findings in SSc skin, another group observed lower SIRT1 expression in skin tissues from patients with hypertrophic scars [103].

Decreased SIRT3 levels and activity were observed in fibrotic areas of SSc and IPF lung tissues and SSc skin biopsies and explanted fibroblasts compared to controls [111, 112]. Moreover, there was a significant negative correlation between SIRT3 levels within lesional skin and disease duration. Reciprocal increases in acetylated MnSOD, a known target of SIRT3 deacetylase activity and marker of reduced SIRT3 activity, have also been shown in skin and lung of SSc patients compared to healthy controls [111] and type 2 alveolar epithelial cells (AT2) in explanted IPF lung tissues [114]. A recent study assessed the expression of all seven SIRTs in lung tissues and fibroblasts from patients with SSc and IPF [121]. The authors found a tendency for all seven SIRTs to be decreased in patients with fibrosis, with a particularly notable decrease in SIRT7. Although the specific mechanism for this decline is unknown, several studies have demonstrated downregulation of SIRT mRNA and protein [101, 111–113, 121] levels by TGF- β in human dermal and lung fibroblasts [101, 111–113, 121] and murine skin and lung tissues [101, 112]. Decreased SIRT expression in SSc may be a marker both of aging, given the known decline of SIRTs with age, and TGF- β -driven fibrosis.

Contradicting these findings, increased SIRT1 levels were found in SSc skin fibroblasts [102] and IPF lung tissues [98], as well as in bleomycin-treated mice [98, 102]. Another report showed increased SIRT6 expression in epithelial cells lining dilated cysts in patients with IPF, but not in normal lung, and upregulation of SIRT6 by TGF- β in primary human bronchial epithelial cells [118]. The reasons for such inconsistencies with the majority of data remain to be elucidated, but may depend on differences between specific cell types or specific SIRTs being studied.

Sirtuin regulation of TGF- β effects

Wei et al. reported that SIRT1 activation with resveratrol or selective SIRT1 activators suppressed various profibrotic effects of TGF- β in fibroblasts and organ cultures derived from normal skin. Antifibrotic effects of resveratrol in fibroblasts were overcome by SIRT1 inhibition, suggesting SIRT1 alone is sufficient to attenuate TGF- β -mediated profibrotic responses [100]. Other studies showed similar fibrosis-inhibiting effects mediated by SIRT1 in cultured fibroblasts from patients with hypertrophic scars [103] and transformed human and mouse fibroblast cell lines [99]. Similar to studies on SIRT1, several groups found that SIRT3 inhibited TGF- β -induced profibrotic effects such as increases in collagen, α -smooth muscle actin (α -SMA), or plasminogen activator inhibitor-1 (PAI1) in cultured normal human lung or skin fibroblasts [111–113]. Similar effects were seen in vivo with exaggerated profibrotic responses to bleomycin in SIRT3 KO mice [112–114] and decreased bleomycin-induced lung fibrosis in SIRT3 transgenic mice [113]. Intratracheal adenoviral delivery of SIRT6 similarly suppressed bleomycin-induced epithelial to mesenchymal transition (EMT) in murine lung [119]. SIRT7 has also been identified as a novel regulator of lung fibrosis with suppression of basal and TGF- β -induced profibrotic effects in normal and IPF lung fibroblasts [121].

Contradicting these studies that consistently show suppression by SIRTs of TGF- β -induced profibrotic responses, one study [101] found that SIRT1 activation with resveratrol enhanced numerous TGF- β -induced profibrotic effects via the Smad2/3 pathway in cultured

fibroblasts, and fibroblast-specific SIRT1 knockdown alleviated bleomycin- or TGF- β -induced dermal fibrosis in vivo. The authors argued for an endogenous negative feedback loop in which SIRT1 inhibition by TGF- β serves to limit TGF- β signaling in fibroblasts. They claimed that the modest SIRT1 decrease in SSc skin was insufficient to limit TGF- β -driven fibrosis and that SIRT1 inhibition has the potential to suppress TGF- β signaling and thus ameliorate fibrosis.

Mechanistic contributions of sirtuins to scleroderma

TGF- β signaling—SIRT1, SIRT3, and SIRT7 have been shown to ameliorate fibrosis in the lungs and other organs by inhibiting TGF- β -induced signaling [61, 89, 100, 105, 111, 112, 121]. Wei et al. found that SIRT1 disrupted Smad-dependent TGF- β signaling without affecting phosphorylation or nuclear translocation of Smad2, but suppressed p300, a known critical downstream component of the Smad pathway [100]. Zeng et al. observed a similar potential role for p300 in mediating the effects of SIRT1 on fibrosis in a transformed fibroblast cell line [98]. Bai et al. demonstrated increased fibrosis and Smad2 phosphorylation after SIRT1 shRNA intradermal injections in a mouse cutaneous wound model, implicating the TGF- β /Smad pathway in the regulation of scar formation by SIRT1 [103]. Sosulski et al. demonstrated exaggerated Smad3-dependent fibrotic responses to bleomycin in SIRT3 deficient mice [112], and Akamata et al. showed that SIRT3 overexpression in skin and lung fibroblasts suppressed TGF- β -induced Smad-responsive luciferase activity [111]. Tian et al. showed that SIRT6 interferes with Smad3 signaling at multiple levels to suppress EMT in A459 cells [119]. Similar to studies on SIRT1 and SIRT3, SIRT7 overexpression in fibroblasts from healthy controls and patients decreased Smad2/3 levels, implicating Smad2/3 signaling as a mediator of SIRT7's antifibrotic effects [121].

mTOR pathway—In addition to finding that SIRT1 activation with resveratrol suppressed TGF- β -induced Smad3 phosphorylation and Smad-binding element activity in the collagen promoter, Chu et al. observed that SIRT1 attenuated mTOR activity [99]. Inhibition of mTOR with rapamycin had an antifibrotic effect, indicating that the antifibrotic effect of SIRT1 may be mediated by mTOR inhibition. The authors also considered the importance of inflammation in propelling fibrosis. SIRT1 overexpression or activation with resveratrol in cultured fibroblasts suppressed TNF- α -induced increases in pro-inflammatory cytokine levels as well as increases in NF- κ B transcription and protein acetylation, suggesting that SIRT1's anti-inflammatory effects may involve the NF- κ B pathway.

A subsequent study by this group found increased inflammatory and fibrogenic factors and increased mTOR levels in skin fibroblasts from patients with SSc and lesional skin in bleomycin-challenged mice compared to controls [102]. Activation of SIRT1 with resveratrol had a protective effect in the bleomycin model, which the authors ascribed to downregulation of mTOR and subsequent attenuation of multiple pro-inflammatory and profibrotic responses. Based on their data, the authors suggested a protective feedback loop in which mTOR-dependent anti-inflammatory and antifibrotic regulation by SIRT1 fails in SSc, but can be functionally restored by elevating SIRT1 levels with resveratrol. Persistent activation of the mTOR pathway has also been shown in aged and IPF lung fibroblasts and

linked to inhibition of autophagy, development of cellular senescence, and fibroblast resistance to apoptosis in pulmonary fibrosis [123, 124].

Oxidative stress and mitochondrial DNA damage—SIRT3 suppression by TGF- β has been linked to increased oxidative stress contributing to fibrosis [112]. Bleomycin and TGF- β inactivated mitochondrial antioxidant proteins in mouse lungs by increasing their acetylation, and resveratrol attenuated these effects in human lung fibroblasts (HLF). The authors suggested a negative feedback mechanism whereby TGF- β -induced SIRT3 inhibition results in less SIRT3-dependent deacetylase activity and, consequently, a deficient antioxidant response [112]. Bindu et al. concluded that SIRT3's inhibition of myofibroblast differentiation occurs independently of its antioxidant activity based on the observation that the antioxidant EUK134 failed to suppress α -SMA levels in TGF- β -stimulated normal HLF [113]. SIRT3 overexpression in HLF also suppressed TGF- β -induced mitochondrial DNA (mtDNA) damage and decreases in the mtDNA base excision repair enzyme 8-oxoguanine-DNA glycosylase-1 (OGG1), an effect not seen with EUK134 pre-treatment. The authors interpreted this latter finding as evidence that TGF- β -induced SIRT3-downregulation, and not ROS production, contributes to decreased OGG1 expression. Furthermore, SIRT3 overexpression in OGG1-silenced HLF failed to suppress TGF- β -induced increases in α -SMA, suggesting prevention of mitochondrial DNA damage by stabilizing OGG1 as a mechanism for SIRT3's antifibrotic effect. A similar mechanism for SIRT3 protection was observed in the bleomycin model of lung fibrosis.

Jablonski et al. investigated SIRT3 regulation of oxidative stress-induced mitochondrial dysfunction and apoptosis in asbestos-induced lung fibrosis [114]. Oxidative stress induced by asbestos or hydrogen peroxide decreased SIRT3 protein levels and, to a greater extent, SIRT3 activity in epithelial cell lines and rat primary AT2 cells as shown by elevated levels of acetylated total mitochondrial protein, MnSOD, or OGG1. SIRT3 overexpression in A549 cells suppressed acetylated MnSOD, CC-9, a marker of apoptosis, and mitochondrial DNA damage. Conversely, compared to WT mice, SIRT3 KO mice showed increased markers of apoptosis in lung tissues and increased mitochondrial DNA damage in isolated primary AT2 cells at baseline and in response to asbestos-induced oxidative stress. The authors proposed a model where loss of SIRT3 increases susceptibility to lung fibrosis by augmenting oxidant-induced mitochondrial protein acetylation, mtDNA damage, and apoptosis. They acknowledged that SIRT3's antifibrotic effects may occur by alteration of other mitochondrial targets, which is supported by a recent proteomic analysis showing SIRT3 interacts with numerous acetylated mitochondrial proteins and may maintain mitochondrial homeostasis under stress conditions [125].

Cellular senescence—Minagawa et al. investigated the role of SIRT6 in regulating the profibrotic effects of senescent epithelium in patients with IPF. The authors found increased markers of senescence (β -galactosidase and the senescence-associated cyclin-dependent kinase inhibitor p21/waf-1) in lung epithelium from patients with IPF compared to patients with COPD or controls [118]. SIRT6 was increased and co-localized with p21 in IPF lung cysts and attenuated TGF- β -induced increases in cellular senescence in human bronchial epithelial cells (HBEC) by inhibiting p21 via proteosomal degradation. SIRT6

overexpression reduced IL-1 β secretion in TGF- β -induced senescent HBEC, and primary lung fibroblasts cultured in media from senescent HBEC produced less collagen than fibroblasts cultured in control HBEC-conditioned media, an effect that was suppressed in the presence of an IL-1 β receptor antagonist. Taken together, these results suggest a model where IL-1 β secreted by TGF- β -induced senescent epithelium promotes fibrosis, and SIRT6 intercepts this profibrotic process by inhibiting both cellular senescence and IL-1 β expression in HBEC. The authors suggested that SIRT6 upregulation in HBEC may be an insufficient compensatory mechanism against stress-induced cellular senescence in IPF.

Senescent phenotype of lung fibroblasts in pulmonary fibrosis—Fibrosis is believed to result from proliferation and activation of α -SMA-expressing myofibroblasts, which produce excessive extracellular matrix and secrete profibrotic factors [126]. Recently, a new paradigm that helps explain the increased prevalence of fibrosis with aging has emerged, suggesting that fibrosis results from the accumulation and decreased turnover of senescent fibroblasts. Fibroblasts from patients with IPF have decreased proliferation and apoptosis than normal fibroblasts, are more resistant to oxidative stress, and exhibit morphological features of cellular senescence [127, 128]. Markers of cellular senescence such as β -galactosidase, p16, and p21 are increased in fibroblasts from patients with IPF and in murine lung fibrosis [128, 129], and the secretome (SASP) of senescent fibroblasts is profibrotic [129]. Compared to aged matched controls, IPF lung fibroblasts have shorter telomeres, mitochondrial dysfunction, and increased collagen production and endoplasmic reticulum stress in response to TGF- β [128]. New therapeutic strategies for fibrosis such as senolytics targeting senescent fibroblasts are emerging and have shown to improve lung function and exercise capacity in bleomycin-mouse models [129]. SIRT3 and SIRT5 depletion have also been shown to induce senescence in human fibroblasts [130]. Decreased SIRT expression in SSc may contribute to fibrosis by participating in the development of fibroblast senescence.

Therapeutic implications

Important for the development of future therapies, SIRT1 activation with resveratrol suppresses collagen levels in cultured SSc skin fibroblasts [100, 102] and attenuates skin [102] and lung [98, 99] fibrosis and inflammation [99] in the bleomycin mouse model. Resveratrol treatment in mice prior to bleomycin had a more substantial effect than simultaneous or delayed treatment on reducing inflammatory responses and dermal thickness, suggesting that SIRT1 may play a preventative therapeutic role in early SSc [102].

Akamata et al. investigated the role of a new synthetic compound, hexafluoro, in fibrosis through its modulation of SIRT3 expression. Hexafluoro increased SIRT3 levels in fibroblasts in a time- and dose-dependent fashion, reversed TGF- β -induced SIRT3 suppression, and attenuated TGF- β -induced profibrotic effects. Hexafluoro's antifibrotic effects occurred through suppression of Smad2/3 and STAT3 signaling and only partially in embryonic fibroblasts lacking SIRT3, suggesting at least partial dependence on SIRT3. Hexafluoro also blocked TGF- β -induced increases in ROS and mitochondrial membrane potential. Hexafluoro had similar effects in vivo, improving bleomycin-induced skin and lung fibrosis in mice.

Conclusion

Studies have recently emerged on the contribution of SIRT6 to regulating skin and lung fibrosis in SSc. Mechanisms involved in the interplay between SIRT6, aging, SSc, and fibrosis are summarized in Figure 1. Although a degree of controversy exists in this rapidly expanding field, the majority of data show that SIRT6 attenuate fibrosis in skin and lung fibroblasts and the bleomycin mouse model through multiple mechanisms. Many of these involve TGF- β , either directly through SIRT6 interference with Smad2/3 signaling, or indirectly through SIRT6 suppression of TGF- β -induced pro-fibrotic, aging-associated mechanisms such as oxidative stress, mitochondrial dysfunction, and cellular senescence. Reports have also shown that SIRT6 regulate other highly conserved canonical pathways including mTOR signaling. SIRT6 play a critical role in multiple biologic processes and diseases due to their ubiquitous presence in tissues, distinct subcellular localization, and interaction with a vast array of molecular targets. Future studies will undoubtedly reveal novel mechanisms for this diverse group of molecules in fibrosis and may lead to development of SIRT6-based therapies for SSc and other conditions of premature aging.

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Figure 1.
Cellular and molecular processes linking SIRT6, aging, and SSc.

Table 1

Selected recent literature on the involvement of SIRT6 in organ fibrosis

Sirtuin	Heart	Kidney	Liver	Lung	Skin
SIRT1	[65, 66, 86–88]	[89–95]	[14, 96, 97]	[98, 99]	[100–103]
SIRT2	[104]				
SIRT3	[61, 67, 68, 105–108]	[61, 109, 110]	[61]	[61, 111–114]	[111]
SIRT6	[69, 115]	[116]	[113, 117]	[118, 119]	
SIRT7	[120]			[121]	

Table 2

Selected recent literature on mechanisms involved in the antifibrotic action of SIRT6. Other mechanisms include modulation of telomeres [122], mitophagy [68, 107], apoptosis [66, 120], and metabolism [96, 104].

Sirtuin	TGF- β /Smad3/p300 fibrotic pathway	Oxidative stress or Mitochondrial function	Inflammation
SIRT1	[14, 89, 94, 98–101]	[65, 86, 87, 90, 91]	[93, 99, 102]
SIRT3	[61, 105, 111, 112]	[67, 106, 107, 109, 111–114]	
SIRT6	[118, 119]	[117]	[13, 115]
SIRT7	[121]	[120]	[120]